Title	Comparison of 4 Serological Tests-Complement Fixation, Neutralization, Fluorescent Antibody to Membrane Antigen and Immune Adherence Hemagglutination-for Assay of Antibody to Varicella-Zoster (V-Z) Virus
Author(s)	Yamada, Akira; Ogino, Satoshi; Asano, Yoshizo et al.
Citation	Biken journal : journal of Research Institute for Microbial Diseases. 1979, 22(2), p. 55-60
Version Type	VoR
URL	https://doi.org/10.18910/82552
rights	
Note	

BIKEN JOURNAL Vol. 22 55-60, 1979

COMPARISON OF 4 SEROLOGICAL TESTS—COMPLEMENT FIXATION, NEUTRALIZATION, FLUORESCENT ANTIBODY TO MEMBRANE ANTIGEN AND IMMUNE ADHERENCE HEMAGGLUTINATION—FOR ASSAY OF ANTIBODY TO VARICELLA-ZOSTER (V-Z) VIRUS

AKIRA YAMADA, SATOSHI OGINO, YOSHIZO ASANO, TERUMASA OTSUKA and MICHIAKI TAKAHASHI

Department of Virology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565, Japan

KOICHI BABA and HYAKUJI YABUUCHI

Department of Pediatrics, School of Medicine, Osaka University, Fukushima-ku, Fukushima, Osaka 553, Japan

(Received December 15, 1978)

SUMMARY Four tests for antibody to varicella-zoster (V-Z) virus were compared; these were tests of complement fixation (CF), neutralization (NT), fluorescent antibody to membrane antigen (FAMA) and immune adherence hemagglutination (IAHA). Fifty-two sera from patients with varicella and zoster and from recipients of live varicella vaccine were examined by the 4 tests. The CF test was least sensitive, but the antibody titers by the NT, FAMA and IAHA tests were roughly comparable. The IAHA test was the simplest and fastest to perform, and appeared suitable for routine serological assay to V-Z virus.

The correlation between the IAHA antibody titer and susceptibility of individuals to clinical varicella was investigated retrospectively using sera obtained during 2 outbreaks of varicella in an institution for children, where all the unvaccinated children had developed varicella symptoms. Most of the 25 pre-exposure sera from unvaccinated children examined by the IAHA test had titers of $<1:2$. In contrast, all the 23 sera from vaccinated children who did not develop varicella had detectable antibody titers of 1: 2 to 1: 64. These results indicate that the IAHA titer reflects the susceptibility or resistance of individuals to clinical varicella.

INTRODUCTION

The CF test was used first for assay of antibody to V-Z virus, (Schmidt et al., 1964), and the NT test (Caunt and Shaw, 1969) and FAMA test (Williams et al., 1974, Gershon and Krugman, 1975) were introduced later. The IAHA test was developed most recently and there

are reports that it is as sensitive as the FAMA test and that both tests are much more sensitive than the CF test (Gershon et al., 1976, Kalter et al., 1977, Gillani and Spence, 1978). However, the NT test has generally been thought to be the most reliable method for detecting specific antibody, and it has not yet been compared with the IAHA test or FAMA test. We previously described (Asano and Takahashi, 1978) a simplified NT test for V-Z virus. In the present study, we compared the sensitivities and simplicities of the CF, NT, FAMA and IAHA tests for measurement of V-Z antibody in sera from patients with varicella and zoster and from recipients of varicella vaccine.

We also examined the sensitivity of IAHA test for assessing the immune status of individuals to clinical varicella by retrospective examination of sera obtained in 2 outbreaks of varicella in an institution for children.

MATERIALS AND METHODS

1. *Virus*

The Kawaguchi strain of V-Z virus (Takahashi et al., 1975) was used for all serological studies.

2. *Cells*

Human embryonic lung (HEL) cells at the 10th to 20th passage were used. Cells were grown in a mixture of equal volumes of Eagle MEM and Medium 199, supplemented with 10% calf serum. Cultures were maintained in similar medium, but with 3% calf serum.

3. *Complement fixation (CF) test*

CF antigen was prepared as described previously (Asano and Takahashi, 1978). CF antibody titer was measured by the standard microtiter technique, using 4 units of antigen and 2 units of complement, with overnight fixation at 4 C.

4. *Neutralization (NT) test*

The NT test was carried out as described previously (Asano and Takahashi, 1978). Appropriately diluted virus solution (100 to 200 PFU/0.1 ml) was mixed with an equal volume of serial 2-fold dilutions of serum, and the mixtures were incubated at 37 C for 30 min with occasional shaking. Then 0.2 ml volumes of serum-virus mixture were inoculated onto monolayers in wells of plastic dishes (Linbro, FB-6) and these were incubated at 37 C for 2 hr. Then maintenance medium was added, and the number of foci was counted after 6 to 8 days of incubation. Antibody titers were expressed as the reciprocal of the highest dilution of serum producing 50% or more reduction in foci.

5. *Test of fluorescent antibody to membrane antigen (FAMA)*

The FAMA test was performed essentially as described by Williams et al. (1974). Monolayers of HEL cells infected with V-Z virus were scraped into 0.01 M phosphate buffered saline (PBS) containing 0.08% EDTA. The cells were collected by low-speed centrifugation and resuspended in PBS at a concentration of approximately 10^6 cells/ml, and 0.025 ml of the cell suspension was incubated with an equal volume of serial dilutions of test serum, starting from 1 : 4 dilution, in microtiter plates (Linbro, V-plate). After incubation for 30 min at room temperature in a moist chamber, the cells were washed 3 times with PBS by centrifugation of the microtiter plates at 2,000 rpm for 10 min. The supernatant was discarded, an appropriate dilution of fluorescein-labeled goat antiserum against human gamma-globulin (Hyland) was added to the washed cells in the well (0.025 ml/well), and they were then incubated for 30 min at room temperature. The cells were washed three times more with PBS. Then about 10 μl of cell suspension was added to one drop of a 1 : 9 solution of PBS and glycerol on a glass slide. A cover slip was placed over the cells and the slides were examined with a fluorescence microscope.

6. *Immune adherence hemagglutination (IAHA) test*

The IAHA test was performed as described by Gershon et al. (1976). Serial dilutions of heat-inactivated serum were made in Linbro microtiter V plates (0.025 ml/well). Dilutions were made in duplicate for V-Z virus antigen and the antigen control in 0.1% gelatin Veronal buffer (GVB, pH 7.4). Volumes of 0.025 ml of V-Z antigen or antigen control were added to dilutions of serum. Plates were placed on a microshaker for 10 sec and then incubated for 1 hr at 37 C. Subsequently 0.025 ml of guinea pig complement was added, usually at

a dilution of 1 : 100. The plates were agitated for 10 sec and then incubated for 40 min at 37 C. DTT-VB-EDTA (0.025 ml, consisting of 1.5 g of dithiothreitol in 500 ml of 0.04 M EDTA in Veronal buffer without gelatin) was added to all wells and the plates were shaken for 10 sec.

7. *Sera*

All sera were stored at −20 C until use and heat-inactivated (56 C for 30 min) before use.

8. *Outbreaks of varicella in an institution for children*

The first outbreak of varicella in an institution for children (of under 2 years old) occurred from December 1975 to March 1976 (Baba et al., 1978). During this outbreak, all 43 unvaccinated children developed typical varicella symptoms while only 8 of 33 children who had just been vaccinated developed symptoms, and these were mild. The second outbreak of varicella in the same institution occurred from December 1977 to March 1978. All the 13 unvaccinated children without a history of varicella developed clinical varicella during this outbreak, while 14 children who had been vaccinated 1 to 2 years previously showed no symptoms.

RESULTS

1. *Comparison of V-Z antibody titers of sera from patients of varicella, zoster and recipients of varicella vaccine by the CF, NT, FAMA and IAHA tests*

The V-Z antibody titers of 52 sera from patients with varicella, zoster and recipients of varicella vaccine are shown in Fig. 1A, B, C, D, E, F. The titers by the CF test were generally lower than those by the NT, FAMA, and IAHA tests. The titers by the NT, FAMA and IAHA tests were generally comparable, but those by the IAHA test were usually highest at low serum dilutions.

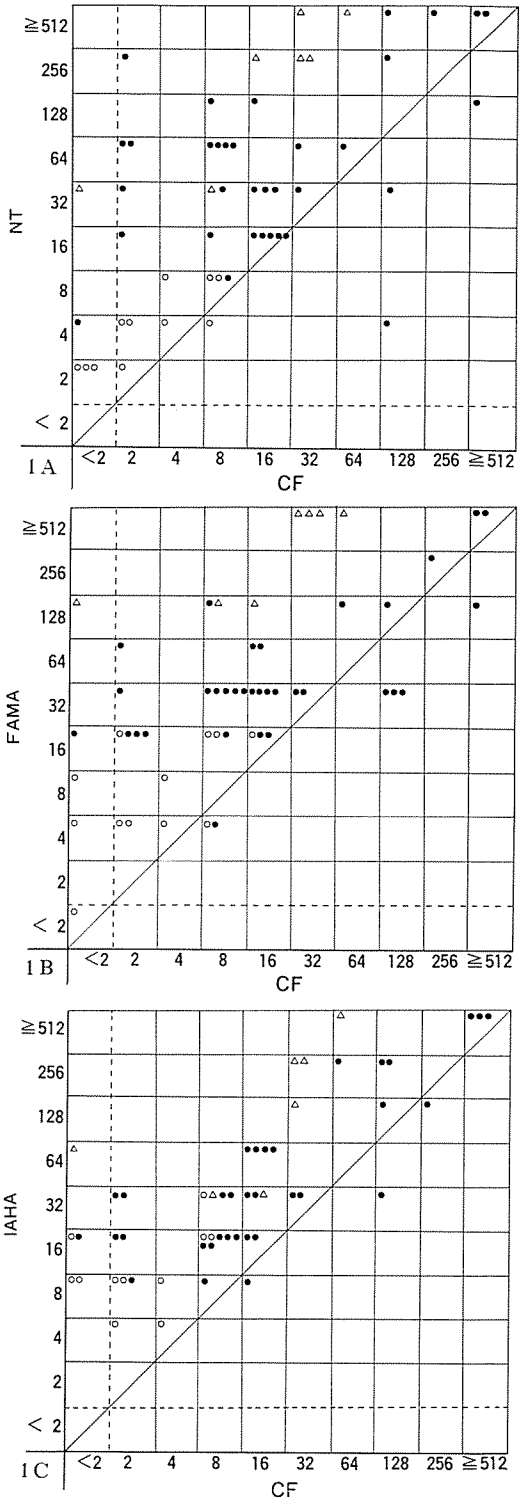
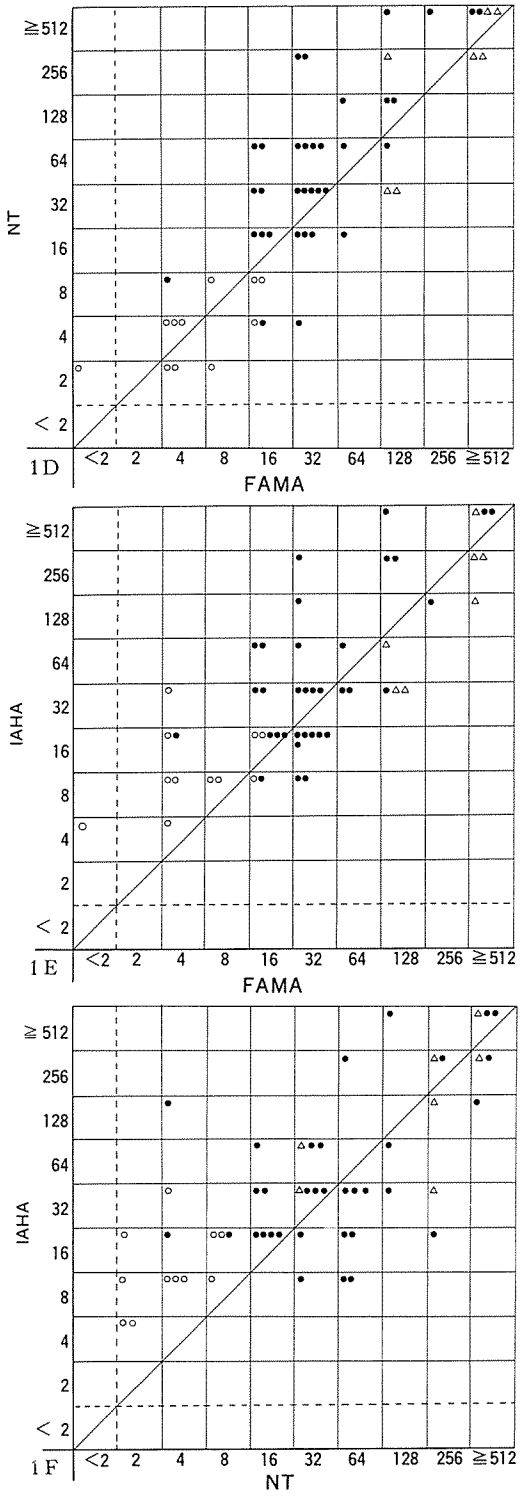


FIGURE 1. Comparison of titers of antibody to varicella-zoster virus by CF, NT, fluorescent antibody to membrane antigen (FAMA) and immune adherence hemagglutination (IAHA) test. symbols: ○, Recipients of live varicella vaccine; △, Patients with varicella; ●, Patients with zoster



2. *Relation of IAHA antibody titers to susceptibilities of individuals to clinical varicella*

Since the IAHA test was the simplest and fastest of the 4 serological tests, the relationship of the IAHA antibody titers to the susceptibilities of individuals to clinical varicella was examined using sera obtained during 2 outbreaks of varicella in an institution for children. These sera included 25 pre-exposure sera from children who developed clinical varicella in the first outbreak. The titer of 23 of these were $<1:2$, while the other 2 gave nonspecific reaction titers of 1:2. The nonspecific reaction could easily be differentiated by reading the control reaction. Twenty-five children had been vaccinated at the onset of the outbreak and were resistant to clinical varicella during the outbreak. Serum specimens obtained 1 month after vaccination were available from 17 of these 25 children and their IAHA titers were found to be 1:2

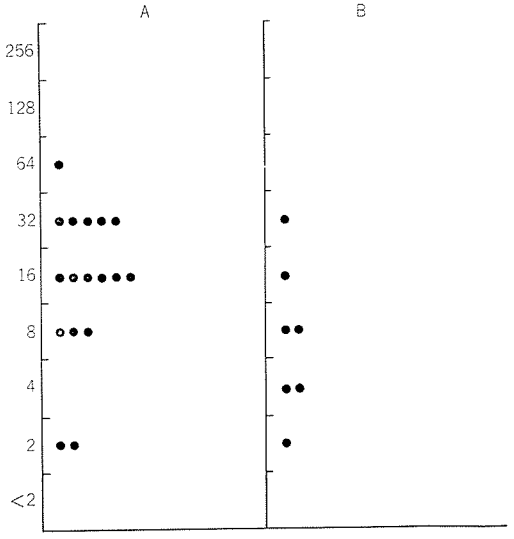


FIGURE 2. IAHA antibody of vaccinated children who were then resistant to clinical varicella infection. (A) IAHA antibody titer of children who were vaccinated 1 month before the 1st outbreak of varicella and resistant to clinical varicella infection. (B) IAHA antibody titer of children who were vaccinated 1 to 2 years before the 2nd outbreak of varicella and resistant to clinical varicella infection.

to 1: 64 (Fig. 2A).

During the second outbreak of varicella in the same institution, which occurred 2 years later, all the 13 unvaccinated children without a history of varicella developed varicella symptoms, while 14 children who had been vaccinated 1 to 2 years before developed no symptoms. Sera of 6 of the 14 children, fortuitously taken 4 months before the outbreak, were available for the IAHA test, and their antibody titers were found to be 1: 2 to 1: 32 (Fig. 2B). These results indicate that the presence of IAHA antibody at a titer of as low as 1: 2 is closely correlated with the protection from clinical varicella infection.

DISCUSSION

The CF test is routinely used for assay of antibody to V-Z virus. However, since CF antibody usually decreases rapidly to an undetectable level after infection, this test has been thought to be unsuitable for examining the immune status of individuals to V-Z virus (Gold and Bodek, 1965). The NT test has generally been regarded as the most reliable method for detecting specific antibody and we have described a simplified NT test (Asano and Takahashi, 1978). But this test requires cell-free virus, which is not easy to obtain, and even the simplified method is laborious, taking several days.

In the present study, comparison of the CF, NT, FAMA and IAHA tests for assay of antibody to V-Z virus showed that the FAMA test was as sensitive as the NT test. The FAMA test is simpler and more rapid than the NT test, but it has the drawback that it requires facilities for tissue culture. Furthermore, it is occasionally difficult to differentiate specific and nonspecific staining at low serum dilutions. The IAHA test is based on the reaction between antigen and antibody complex with complement resulting in activation of the C3 component of complement, which finally leads to interaction with receptor sites located on human erythrocytes. This method has recently been introduced for assay of V-Z antibody and it is reported to be comparable in sensitivity to the FAMA test (Kalter et al. 1977). In the present study, the IAHA test was found to be comparable with the NT test in sensitivity and to be more sensitive than the NT test at low serum dilutions.

Since the IAHA test is the simplest and fastest, and yet is as sensitive as the NT test or FAMA test for detecting antibody, the correlation between the IAHA antibody titer and the susceptibility of individuals to clinical varicella infection was investigated using available sera from children in an institution obtained during outbreaks of varicella infection. Results showed that sera of most children who were susceptible to varicella infection had IAHA antibody titers of $<1:2$, although a few gave a nonspecific reaction. Furthermore all the children with an IAHA antibody titer of 1: 2 to 1: 32 after vaccination were resistant to varicella infection. Thus the presence of detectable IAHA antibody appears to be closely correlated with protection from clinical varicella. However, the absence of detectable IAHA antibody in individuals may not necessarily mean that these individuals have no protection. In fact, Kalter et al. (1977) reported that V-Z antibody titers were detectable by the FAMA test but not by the IAHA test in a few persons who were thought to be immune to varicella. Despite these limitations, the IAHA test appears to be the best routine laboratory test because it is simple and sensitive.

REFERENCES

Asano, Y., and M. Takahashi. 1978. Studies on neutralization of varicella-zoster virus and serological follow-up of cases of varicella and zoster. Biken J. 21: 15–23.

Baba, K., H. Yabuuchi, H. Okuni and M. Takahashi. 1978. Studies with the live varicella vaccine and inactivated skin test antigen: Protective effect of the vaccine and clinical application of the skin test. Pediatrics. 61: 550–555.

Caunt, A. E., and D. G. Shaw. 1969. Neutralization test with varicella-zoster virus. J. Hyg. 67: 343–352.

Gershon, A. A., Z. C. Kalter and S. Steinberg. 1976. Detection of antibody to varicella-zoster virus by immune adherence hemagglutination. Proc. Soc. Exp. Biol. Med. 151: 762–765.

Gershon, A. A., and S. Krugman. 1975. Seroepidemiologic survey of varicella: value of specific fluorescent antibody test. Pediatrics. 56: 1005–1008.

Gillani, A., and L. Spence. 1978. Immune adherence hemagglutination test applied to the study of herpes simplex and varicella-zoster virus infections. J. Clin. Microbiol. 7: 114–117.

Gold, E., and B. Bodek. 1965. Complement fixation studies with varicella-zoster antigen. J. Immunol. 95: 692–695.

Kalter, Z. C., S. Steinberg and A. A. Gershon. 1977. Immune adherence hemagglutination: Further observations on demonstration of antibody to varicella-zoster virus. J. Infect. Dis. 135: 1010–1013.

Schmidt, N. J., E. H. Lennette, C. W. Shon and T. T. Shinomoto. 1964. A complement-fixing antigen for varicella-zoster derived from infected cultures of human fetal diploid cells. Proc. Soc. Exp. Biol. Med. 116: 144–149.

Takahashi, M., Y. Okuno, T. Otsuka, J. Osame, A. Takamizawa, T. Sasada and T. Kubo. 1975. Development of a live attenuated varicella vaccine. Biken J. 18: 25–33.

Williams, V., A. Gershon and P. A. Brunell. 1974. Serologic response to varicella-zoster membrane antigens measured by indirect immunofluorescence, J. Infect. Dis. 130: 669–672.